Supplementary Information

Mesoporous ZnO nanocapsules for the induction of enhanced antigen-specific immunological responses.

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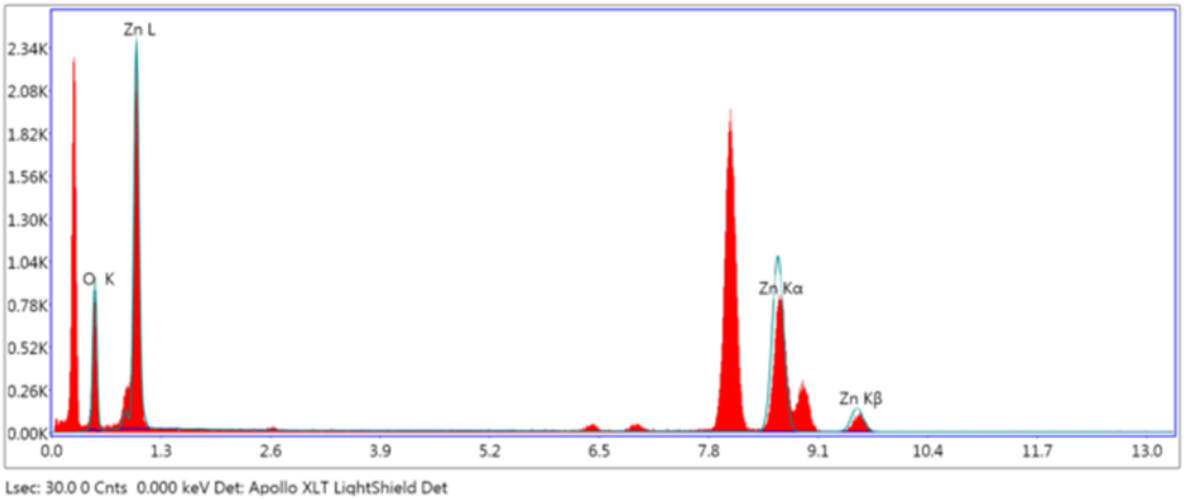
‡ Sumbul Afroz and Himadri Medhi have contributed equally to this work.



Element	Weight %	Atomic %	Net Int.	Error %
O K	94.69	98.65	180.3	3.09
ZnK	5.31	1.35	587.76	10.13

Fig. S1 EDX analysis of mesoporous ZnO nanocapsules with atomic weight % of elements.

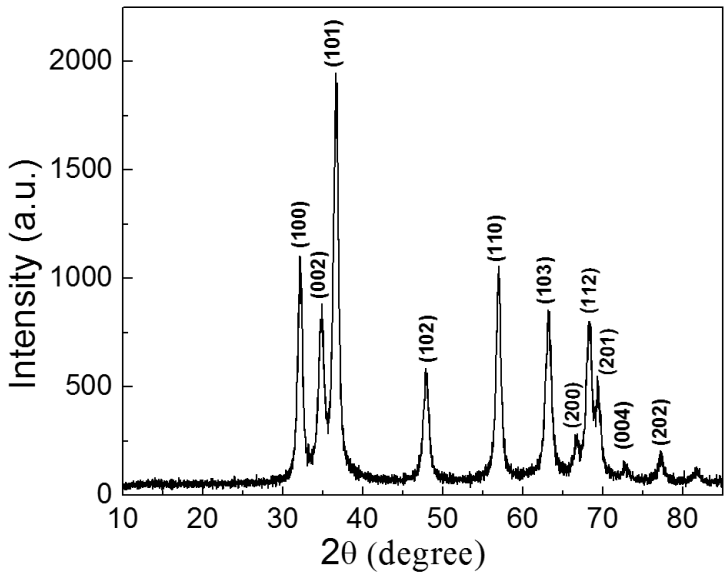


Fig. S2 XRD pattern of mZnO nanocapsules.

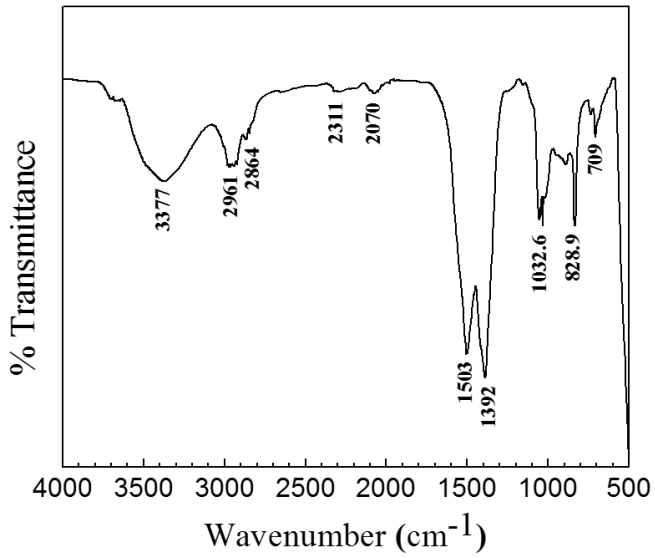


Fig. S3 FTIR analysis of mZnO nanocapsules.

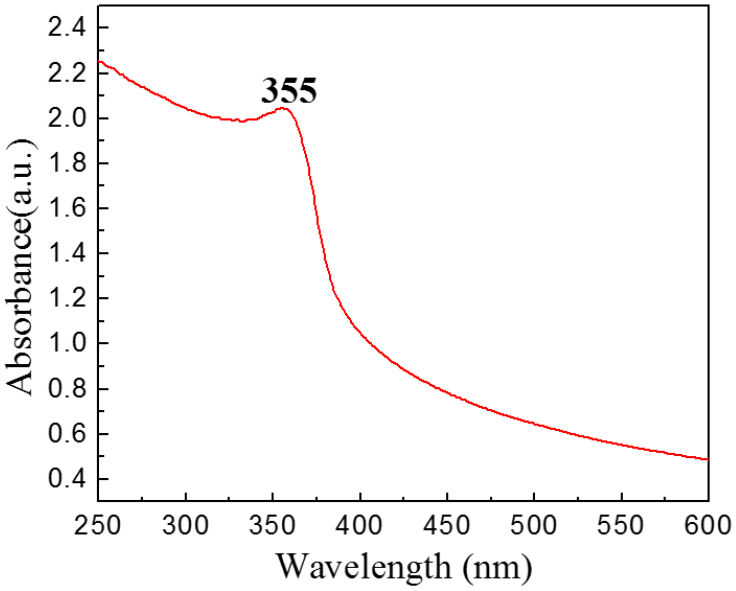


Fig. **S4** UV-Vis absorbance spectra of mZnO nanocapsules

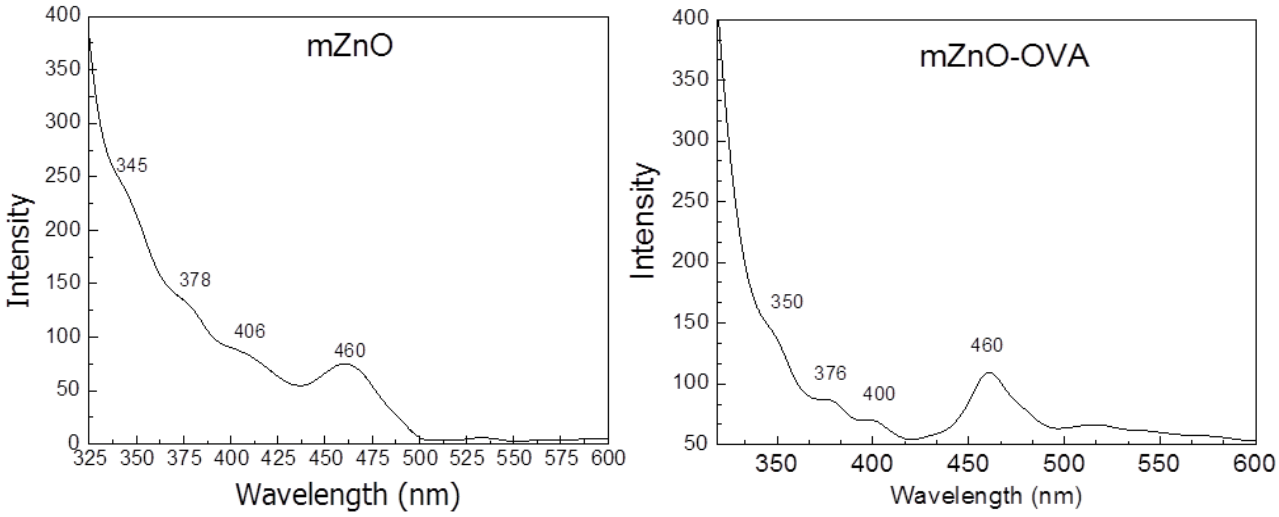


Fig. S5 PL spectra of mZnO and mZnO-OVA nanocapsule

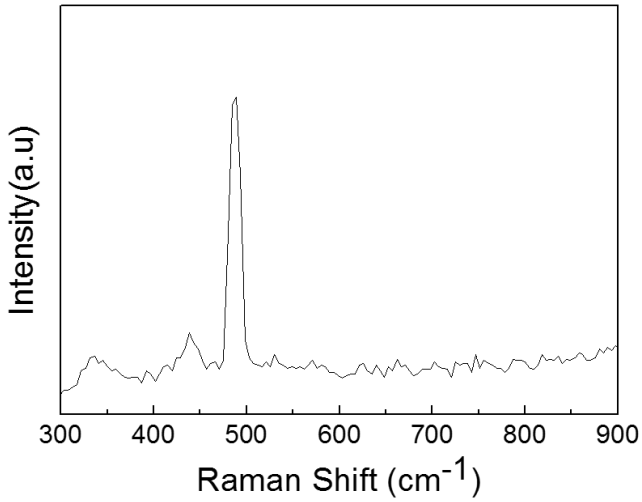


Fig. S6 Raman spectra of mZnO nanocapsules.

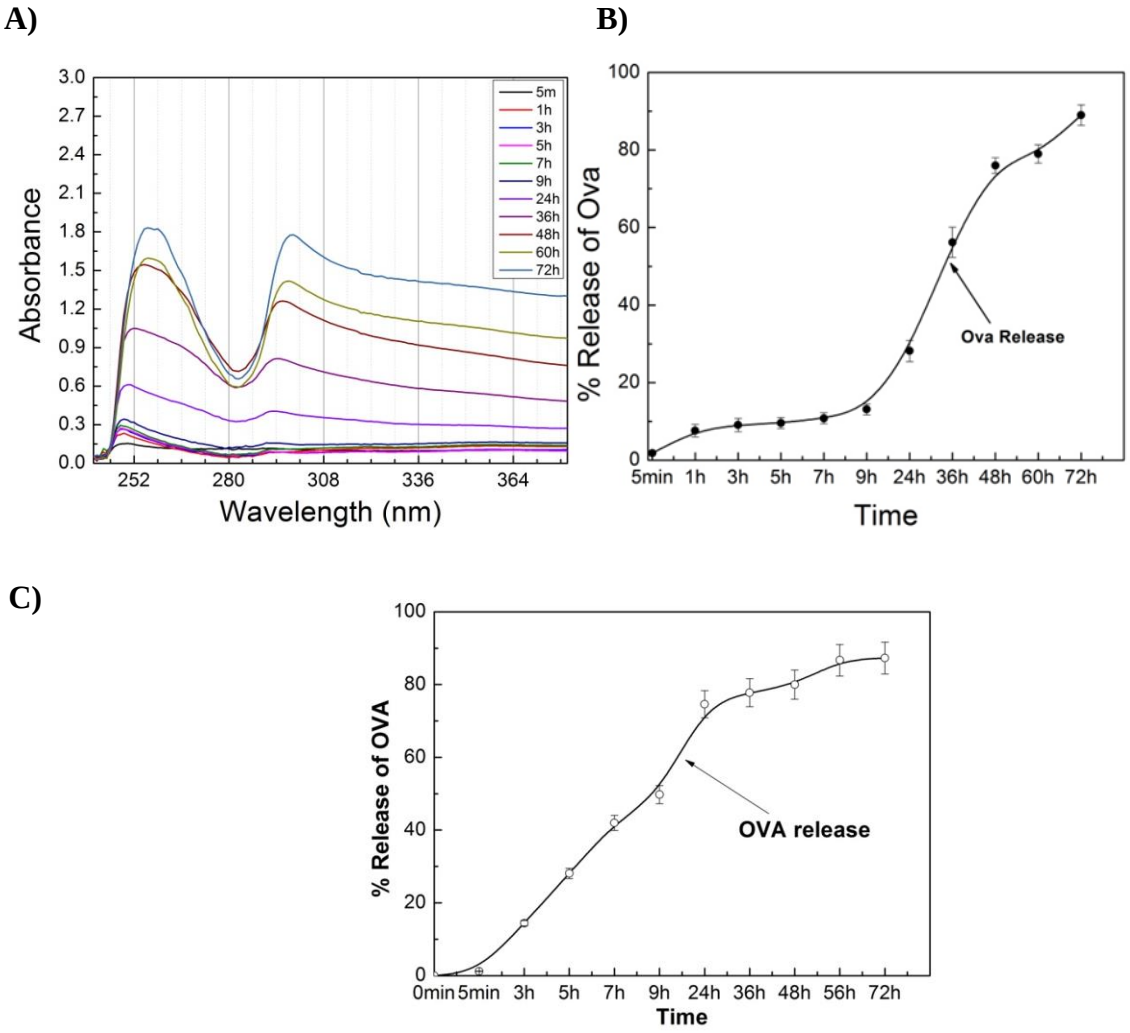
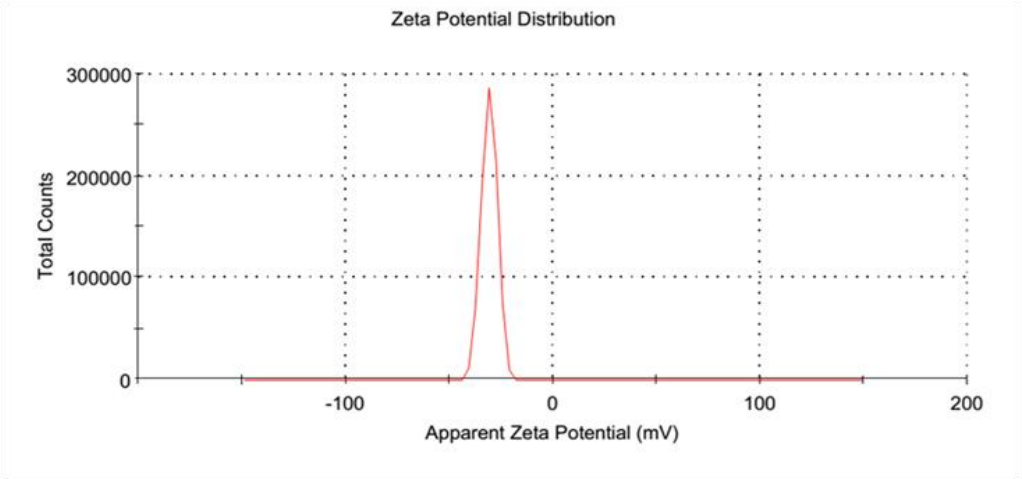


Fig. S7 (A) UV full spectra of time dependent Ova release in culture media and **(B)** Normalized spectrum of percent release of Ova from mZnO(Ova) nanocapsule in culture media **(C)** Normalized spectrum of percent Ova release from mZnO (Ova) nanocapsules in PBS.

Sample 1:

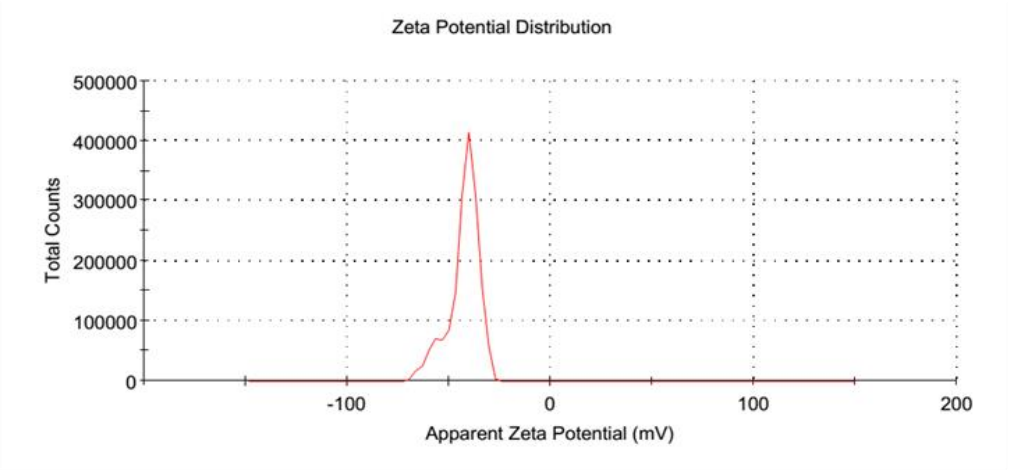
A)

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-30.8	**Peak 1:**	-30.8	100.0	3.84
Zeta Deviation (mV):	3.84	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0225	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				



B)

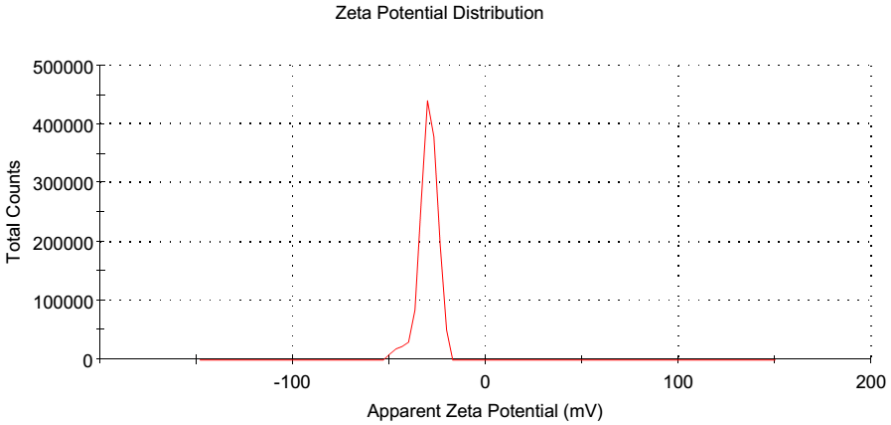
			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-42.8	**Peak 1:**	-40.9	86.7	5.40
Zeta Deviation (mV):	7.69	**Peak 2:**	-58.0	13.3	4.18
Conductivity (mS/cm):	0.0745	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				



Sample2:

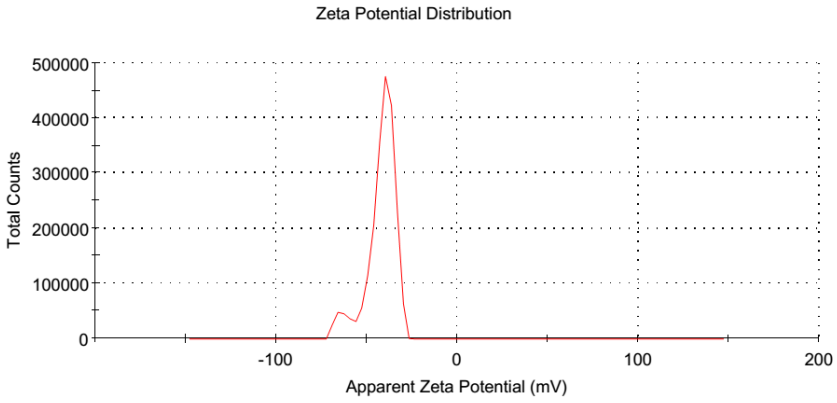
A)

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-30.0	**Peak 1:**	-30.0	100.0	5.12
Zeta Deviation (mV):	5.12	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0225	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				



B)

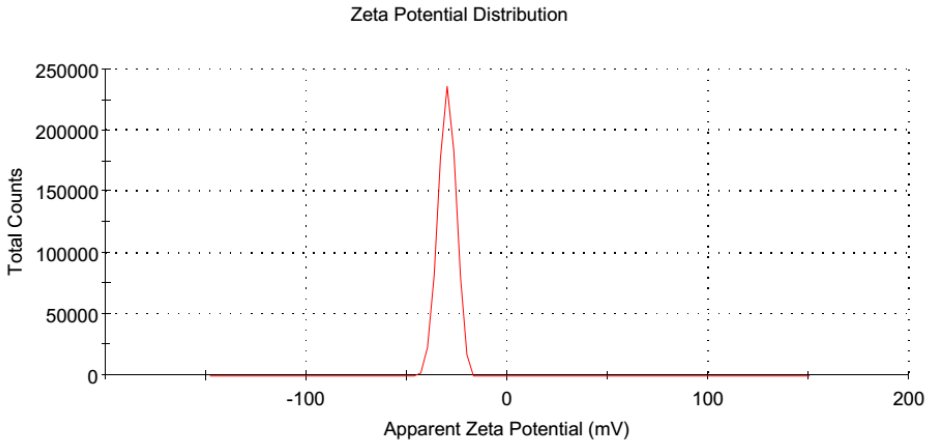
			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-42.1	**Peak 1:**	-40.4	91.2	5.60
Zeta Deviation (mV):	8.23	**Peak 2:**	-62.6	8.8	4.22
Conductivity (mS/cm):	0.0748	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				



Sample 3:

A)

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-30.2	**Peak 1:**	-30.2	100.0	4.34
Zeta Deviation (mV):	4.34	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0227	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				



B)

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-40.9	**Peak 1:**	-36.9	75.7	4.99
Zeta Deviation (mV):	8.91	**Peak 2:**	-54.2	24.3	4.44
Conductivity (mS/cm):	0.0749	**Peak 3:**	0.00	0.0	0.00
Result quality :	Good				

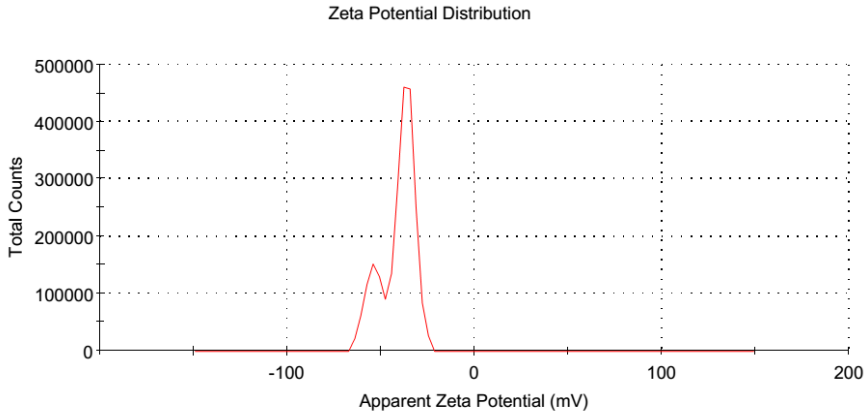


Fig. S8 Zeta potential for three independent samples from three batches of mZnO (A) and mZnO(Ova) (B) nanocapsules.

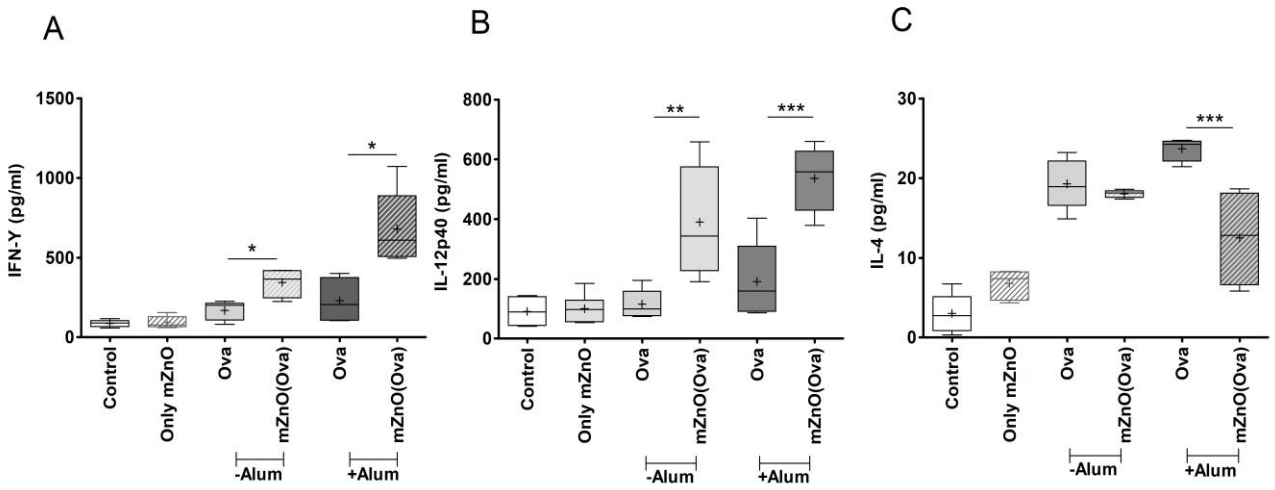


Fig. S9 Th1 cytokines levels were synergistically enhanced in mZnO(Ova) nanocapsules immunized mice after secondary immunization (A-C) IFN-γ, IL-12p40 and IL-4 levels were assessed in Ova(1ug/ml) restimulated splenocytes at 72 hrs post stimulation. Splenocytes were isolated from mice receiving two doses of immunogen, booster at day 14 of primary immunization and sacrificed at day 28 post primary immunization. Graphs represent average cytokine levels. The box plots represent data as mean ± s.e.m. from two independent sets of experiments each with n=5 mice per treatment group per experiment. **P<0.005*P<0.05 (one-way ANOVA with Bonferroni post-hoc test)

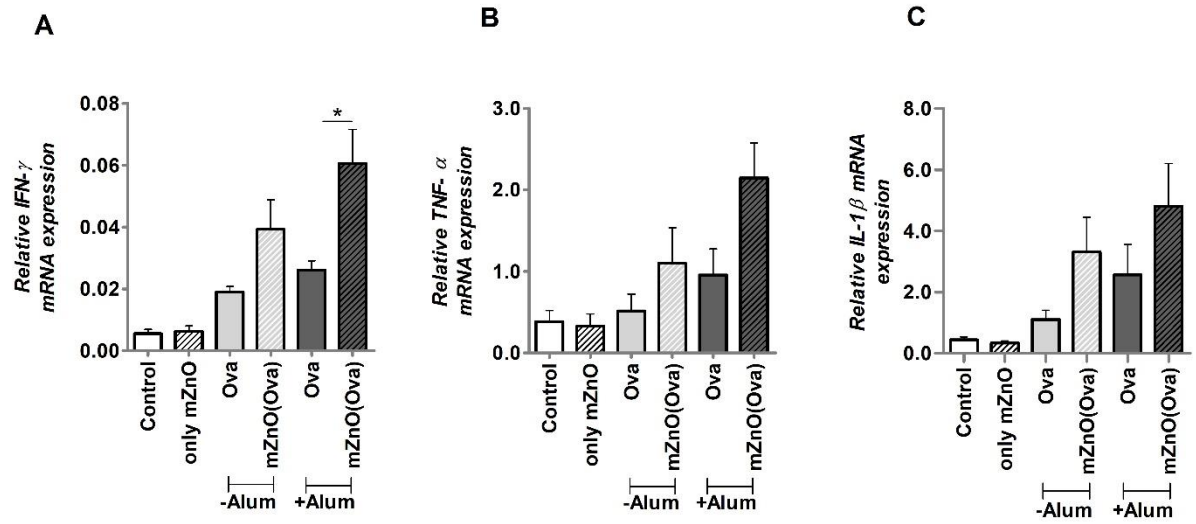
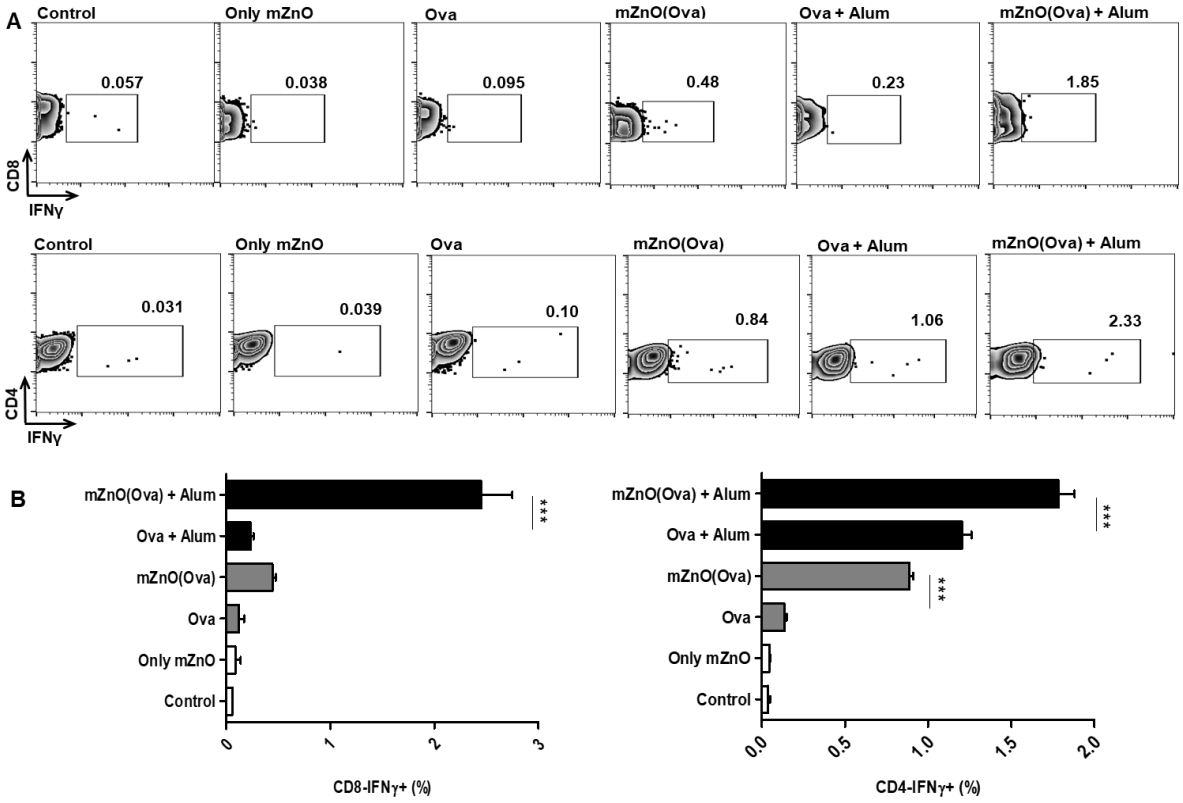


Fig. S10 mRNA expression levels of Th1 cytokines after secondary immunization (A-C) IFN-γ, TNF-α and IL-1β levels were assessed in Ova(1ug/ml) restimulated splenocytes at 36 hrs post stimulation. Splenocytes were isolated from mice receiving two doses of immunogen, booster at day 14 post primary immunization and sacrificed at day 28 post primary immunization. Graphs represent average cytokine expression levels. The bar graphs represent data as mean ± s.e.m. from two independent sets of experiments each with n=5 mice per treatment group per experiment. *P< 0.1 (one-way ANOVA with Bonferroni post-hoc test)

Without booster



With booster

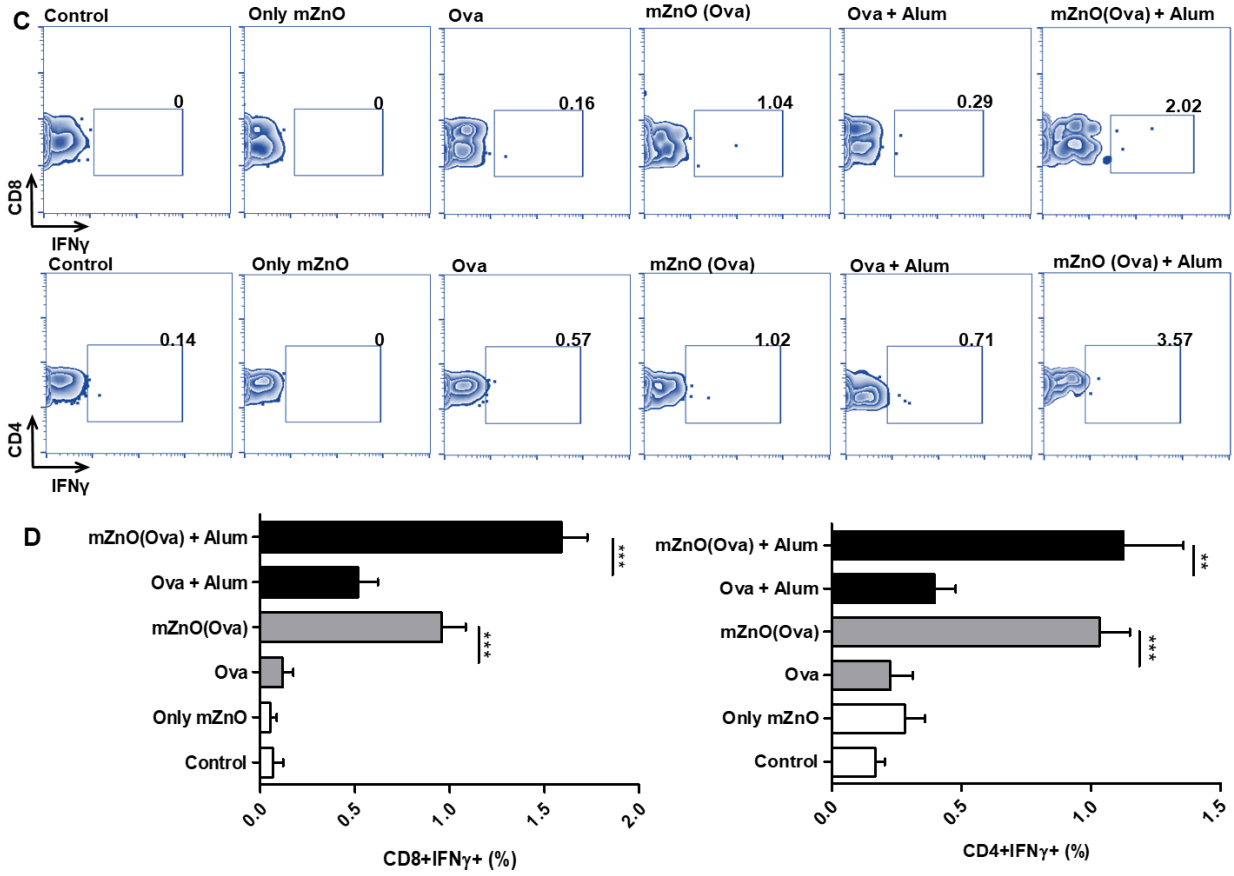


Fig. S11 Antigen specific adaptive T cell responses in PBMCs of immunized mice. (A-B) Mice were immunized as per the indicated groups and were sacrificed at day 21 post immunization. PBMCs were isolated and restimulated with Ova for the identification of IFNγ producing $CD8^+$ and $CD4^+$ T cells. FACS plots for IFNγ expression in $CD8^+$ and $CD4^+$ cells respectively (A). Graphs representing average percentage of $IFN\gamma^+$ cells (B). The bar graphs represent data as mean ± s.e.m. from three independent sets of experiments each with n=4 mice per treatment group per experiment. ***P< 0.001 (one-way ANOVA with Bonferroni post-hoc test). (C-D) Mice were immunized as per the indicated groups, given booster at day 14 post primary immunization and sacrificed at day 28 post primary injection. PBMCs were isolated and restimulated with Ova for the identification of IFNγ producing $CD8^+$ and $CD4^+$ T cells. FACS plots for IFNγ expression in $CD8^+$ and $CD4^+$ cells respectively (C). Graphs representing average percentage of $IFN\gamma^+$ cells (D). The bar graphs represent data as mean ± s.e.m. from two independent sets of experiments each with n=5 mice per treatment group per experiment. ***P< 0.001 (one-way ANOVA with Bonferroni post-hoc test).

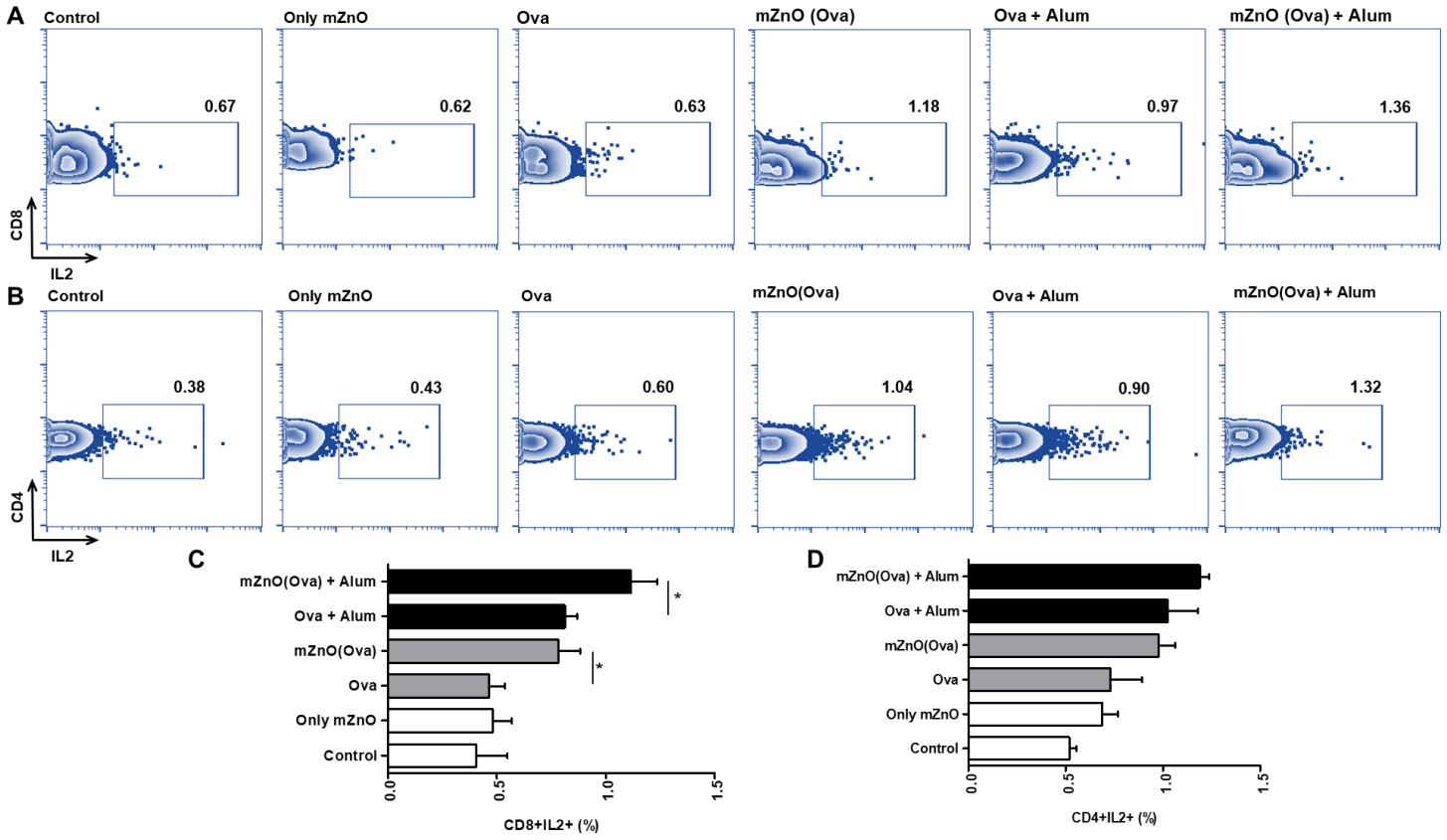


Fig. S12 Antigen specific enhanced levels of IL-2 producing $CD4^+$ and $CD8^+$ T -cells in splenocytes of mZnO(Ova) nanocapsules immunized mice after secondary immunization. (A-D) Mice were immunized as per the indicated groups, given booster at day 14 post primary immunization

and sacrificed at day 28 post primary injection. Splenocytes were isolated and restimulated with Ova for the identification of IL-2 producing $CD8^+$ and $CD4^+$ T cells (A-B) FACS plots for IL-2 expression in $CD8^+$ and $CD4^+$ cells respectively. (C-D) Graphs representing average percentage of IL-2 positive CD8 and CD4 cells. The bar graphs represent data as mean ± s.e.m. from two independent sets of experiments each with n=5 mice per treatment group per experiment. *P< 0.1 (one-way ANOVA with Bonferroni post-hoc test).

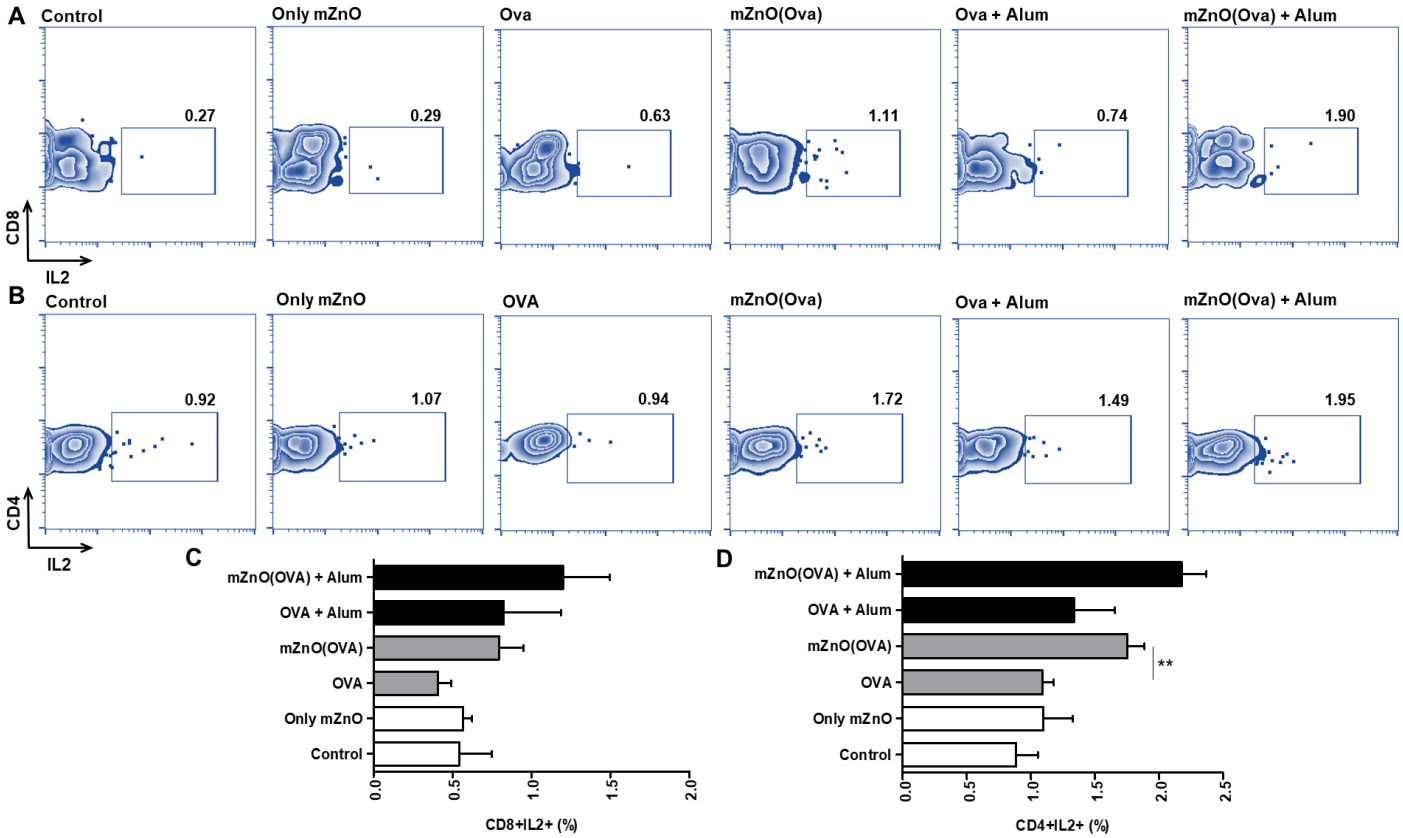


Fig. S13 Antigen specific enhanced levels of IL-2 producing $CD4^+$ and $CD8^+$ T -cells in PBMCs of mZnO(Ova) nanocapsules immunized mice after secondary immunization. (A-D) Mice were immunized as per the indicated groups, given booster at day 14 post primary immunization and sacrificed at day 28 post primary injection. PBMCs were isolated and restimulated with Ova for the identification of IL-2 producing $CD8^+$ and $CD4^+$ T cells (A-B) FACS plots for IL-2 expression in $CD8^+$ and $CD4^+$ cells respectively (C-D). Graphs representing average percentage of IL-2 positive CD8 and CD4 cells. The bar graphs represent data as mean ± s.e.m. from two independent sets of experiments each with n=5 mice per treatment group per experiment. **P< 0.05 (one-way ANOVA with Bonferroni post-hoc test).

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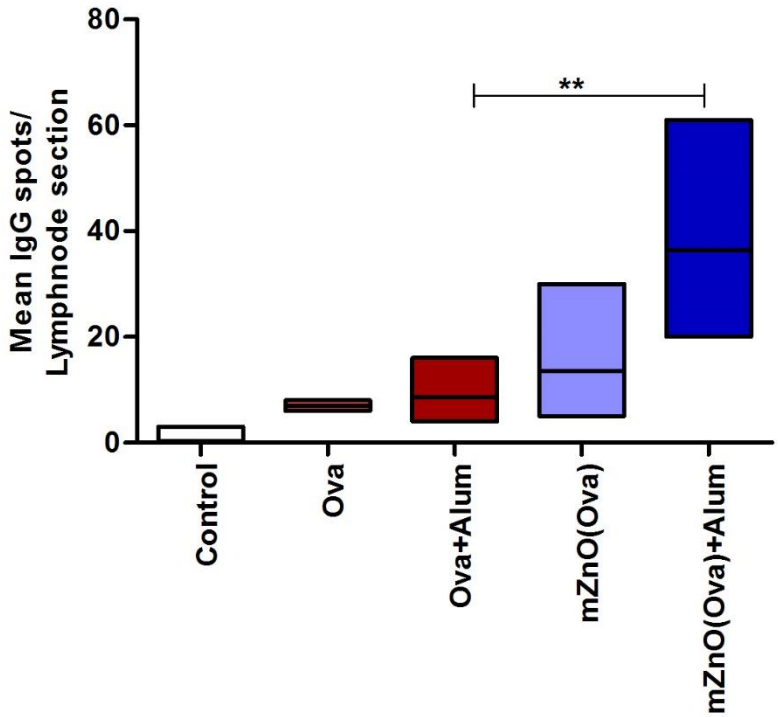

Fig. S14 Ova loaded mZnO nanocapsules generate a higher antigen specific antibody-secreting B- cells. IgG spots were quantified in lymphnode sections from immunized Balb/c mice as per indicated groups. Graph represents mean IgG spots per lymphnode from 2-3 mice per treatment condition per experiment. Images are representative of two independent experiments **P< 0.005 (one-way ANOVA with Bonferroni post-hoc test).